The role of oncostatin M regulates osteoblastic differentiation of dental pulp stem cells through STAT3 pathway

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Abstract Dental pulp stem cells (DPSCs) are a type of mesenchymal stem cells, which have the self-renewal and multi-lineage differentiation potential, including chondrocytes, adipocytes, neural cells and osteoblasts. So they play a significant role in pulp repair and bone regeneration. Oncostatin M (OSM), one of the IL-6 family cytokines, inhibits adipogenic differentiation and stimulates osteogenic differentiation of human bone marrow mesenchymal stem cells. However, the effect of OSM on DPSCs is unclear. We found that OSM induced osteogenic differentiation of DPSCs, promoting matrix mineralization as measured by Alizarin Red S staining. OSM also increased expression of osteogenesis-associated gene products

Alkaline phosphatase, Bone morphogenetic protein 2 (BMP2), Runt-related transcription factor 2 and Osteocalcin (OCN) as assessed by immunoblotting. We also found that OSM activated the Signal Transducer And Activator Of Transcription 3 (STAT3) pathway during the osteogenic differentiation of DPSCs. Blocking the osteogenic differentiation by silencing of STAT3 can significantly inhibit OSM-induced osteogenic differentiation of DPSCs and the expression of related genes, furthermore matrix mineralization was also suppressed. In summary, OSM promotes osteoblastic differentiation of DPSCs and osteogenesis-related genes expression through the JAK3/STAT3 signaling pathway which may be useful for the autologous transplantation of DPSCs.

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Abbreviations

DPSCs	Dental pulp stem cells
MSCs	Mesenchymal stem cells
OSM	Oncostatin M
STAT3	Signal transducer and activator of transcription 3
ALP	Alkaline phosphatase
BMP2	Bone morphogenetic protein 2
Runx2	Runt-related transcription factor 2
OC	Osteogenic differentiation

JAK3	Janus kinase 3
hADSCs	Human adipose mesenchymal stem cells
OCN	Osteocalcin
siJAK3	JAK3 siRNA
siSTAT3	STAT3 siRNA
OSMR	Oncostatin M receptor

Introduction

Dental pulp stem cells (DPSCs) were first isolated from the human pulp tissues (Gronthos et al. 2000). They are known to be pluri-potent cells capable of self-renewal and multilineage differentiation (Gronthos et al. 2002). Recently, DPSCs have become a rapidly developing technology, which can be used in biology and regenerative medicine. DPSCs have the potential to differentiate into several lineages including osteogenic, adipocyte, muscle, neurons and cartilage (Gronthos et al. 2000; Lee et al. 2013; Nakatsuka et al. 2010; Arthur et al. 2008). Comparison of their multipotency with bone marrow stem cells (BMSCs), has demonstrated that proliferation, availability, and cell number of DPSCs were more elevated than for bone marrow MSCs (Huang et al. 2009; Alge et al. 2010). Moreover, DPSCs can easily be isolated from human pulp tissue and do not cause ethical and technical problems (Rodríguez-Lozano et al. 2012). Thus, the determination of the molecular, responsible signal of DPSCs differentiation in the process, may provide a powerful tool for clinicians on the appropriate application of regenerative therapy for the regeneration of these cells.

Oncostatin M (OSM) is an inflammatory factor belonging to interleukin 6 family. It is mainly produced to T lymphocytes and mononuclear cells *in vivo*, and performs many biological activities in the regulation of bone remodeling and is involved in cell growth and differentiation (Gadiant and Patterson 1999; Pflanz et al. 2004; Grant and Begley. 1999; Tanaka and Miyahima 2003; Heymann and Rousselle 2000). OSM is reported to be involved in inflammatory diseases, such as rheumatoid arthritis (Pelletier and Martel-Pelletier. 2003). Furthermore, macrophages produce OSM, and induced alkaline phosphatase (ALP) expression and mineralization of vascular smooth muscle cells, suggesting a key role for this cytokine in atherosclerosis (Shioi et al. 2002). Furthermore, OSM can induce the expression of osteogenic gene products and requires activity of PKCdelta (Smyth et al. 2015). In

addition, OSM promoted the osteogenic differentiation of adipose derived mesenchymal stem cells (Miyaoaka et al. 2006). We have focused on OSM due to its strong anti-adipogenic activity (Malik et al. 1995) and extensive strong ability of bone formation in OSM transgenic mice (Yanai and Obinata. 2001). It has also been reported that OSM is a factor that inhibits adipogenesis and promotes the differentiation of adipose derived mesenchymal stem cells into osteoblasts (Song et al. 2007). However, the precise role underlying OSM-induced osteogenic differentiation of DPSCs is not known.

In our study, we mainly explored the effect of OSM on the osteogenic differentiation of DPSCs and tested the osteogenetic differentiation related gene expression, such as alkaline phosphatase (ALP), Bone morphogenetic protein 2 (BMP2), Runt-related transcription factor 2 (Runx2) and Osteocalcin (OCN). As far as we know, this is the first demonstration of OSM to stimulate the differentiation of DPSCs, and OSM could promoted osteogenic differentiation of DPSCs by activating the JAK3/STAT3 signaling.

Materials and methods

Cell cultures

Normal human impacted third molars were collected from patients 13–23 years of age ($n = 9$) after giving the informed consents which were approved by the Ethics Committee of the Affiliated Hospital of Nantong University. All subjects were free of carious lesions and oral infection. We isolated DPSCs by cleaning the tooth surface, cutting around the cemento-enamel junction using sterilized dental fissure burs and then opening to reveal the pulp chamber. The pulp was digested in a solution of 3 mg/ml collagenase type I for 1 h at 37 °C. Single-cell suspensions were obtained by passing the digested tissues through a 70- μ m cell strainer (BD, NJ, USA). Cell suspensions of dental pulp were seeded into 25 cm² culture dishes and cultured in Dulbecco's modified Eagle medium (DMEM) (Hycone, Logan, UT, USA) supplemented with 10 % fetal bovine serum (FBS) (Gibco, Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin and 100 μ g/ml streptomycin at 37 °C in 5 % CO₂. The medium was changed every 3 days. Cells were passaged at the ratio of 1:3 when they reached 85–90 % confluence. The cell populations were characterized by positive

staining with anti-CD90 (Sigma-Aldrich, St. Louis, MO, USA), anti-CD105 (Sigma-Aldrich, USA), anti-CD146 (Sigma-Aldrich) and STRO-1 (Sigma-Aldrich) and the absence of CD45 (Sigma-Aldrich) (Homayounfar et al. 2016). Cells at passage 3 (P₃) were used in the subsequent experiments.

Osteogenic differentiation

DPSCs were plated at a density of 2×10^4 cells/cm² and cultured in proliferation medium supplemented with 0.1 μ M dexamethasone, 10 mM β -glycerophosphate (Sigma-Aldrich) and 50 μ g/ml ascorbic acid (Sigma-Aldrich). DPSCs were differentiated for 3, 5, 7, 14 days in the absence or presence of OSM (10 ng/ml) (Sigma-Aldrich). The degree of extracellular matrix calcification was estimated using an Alizarin red S (Sigma-Aldrich) and Alkaline phosphatase (SenBeiJia, Nanjing, China) staining.

DPSCs were plated at a density of 2×10^4 cells/cm² and cultured in growth medium supplemented with osteogenic medium for 14 days. 10 ng/ml OSM was added or not. For pharmacological activation of JAK/STAT3 signaling pathway, DPSCs were supplemented with WHI-P154 (inhibitor of JAK3, Sigma-Aldrich) for 14 days.

MTT assay

Dental pulp stem cells were seeded on 96-well plates at a cell density of 1×10^4 cells/well. At 12, 24, 48 and 72 h after the OSM treatment, the proliferation/survival of the cells was evaluated using the methylthiazolyldiphenyltetrazolium bromide (MTT) test. The culture medium was replaced with 5 mg/ml MTT solution in PBS and the plates were incubated for 5 h at 37 °C in air plus 5 % CO₂. The precipitate was extracted with dimethylsulfoxide (DMSO) (Sigma-Aldrich) and the optical density was measured at the wavelength of 550 nm.

Alizarin red S and ALP staining

DPSCs were fixed with 4 % PFA for 1 h and washed with PBS. 2 % Alizarin red S solution was applied and DPSCs were placed in an incubator at 37 °C for 2 h. Mineralization was quantified by extracting the Alizarin red S stain with 100 mM cetylpyridinium chloride (Sigma-Aldrich) at room temperature for 2 h. Absorbance of the extracted Alizarin red S stain was

measured at 570 nm. DPSCs were subjected to ALP staining using the ALP assay kit according to the manufacturer's instructions. Alkaline phosphatase and Alizarin red S activities were determined by a colorimetric assay.

Western blotting

DPSCs were treated with OSM under appropriate conditions, washed with ice-cold PBS, and cells were lysed in lysis buffer consisting of 50 mM TRIS, 150 mM NaCl, 2 % sodium dodecyl sulfate (SDS) and a protease inhibitor mixture. After centrifugation at 12000 rpm for 12 min, protein concentrations were determined using the Bradford assay (Bio-Rad, Hercules, CA, USA). The resulting supernatant (50 μ g of protein) was subjected to SDS-polyacrylamide gel electrophoresis (PAGE). The separated proteins were transferred onto PVDF membranes at 350 mA for 2.5 h in a blotting apparatus (BioRAD). Membranes were blocked with 5 % non-fat milk and the membranes were immunoblotted with various antibodies (1:500) at 4 °C overnight and subsequently with anti-rabbit horseradish peroxidase-conjugated secondary antibodies (1:1000) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 h at room temperature. Concomitantly, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was run as a reference protein. The following primary antibodies were used: anti-GAPDH (anti-rabbit, Santa Cruz Biotechnology), anti-JAK3 (anti-rabbit, Santa Cruz Biotechnology), anti-p-STAT3 (anti-mouse, Santa Cruz Biotechnology), anti-STAT3 (anti-mouse, Santa Cruz Biotechnology), anti-ALP (anti-mouse, Santa Cruz Biotechnology), anti-OCN (anti-mouse, Santa Cruz Biotechnology), anti-BMP2 (anti-mouse, Santa Cruz Biotechnology), anti-Runx2 (anti-mouse, Santa Cruz Biotechnology) antibodies.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis

Total RNA was extracted from cells and reverse transcribed using conventional protocols. PCR amplification was performed using the following primer sets: GAPDH: forward: 5'-CCCTCCAGAAGAAAATGG AT-3', reverse: 5'-ATAGCTCAGATGCTCGCTCA-3';

Runx2: forward: 5'-CTCACTACCACACCTACCTG-3', reverse: 5'-TCAATATGGTCGCCAAACAGATTC-3';

ALP: forward: 5'-TGAAATATGCCCTGGAGC-3', reverse: 5'-TCACGTTGTTCTGTTTAG-3';

BMP2: forward: 5'-CTAGACCTGTATCGCAGGCA-3', reverse: 5'-TTTCCCCTCGTTTCTGGTA-3';

OCN: forward: 5'-GGACACAATGGATTGCAAGG-3', reverse: 5'-TAACCACTGCTCCACTCTGG-3'.

All the primer sequences were determined using established GenBank sequences. The primers were used to amplify the duplicate PCR reactions. Each sample was analyzed in triplicate and GAPDH was used as a control.

siRNA and transfection

JAK3 siRNA (SASI_Hs01_00118130), STAT3 siRNA (SASI_Hs01_00061864) were purchased from Sigma-Aldrich. siRNA was transfected into DPSCs using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. After incubated (at 37 °C and 5 % CO₂) for 6 h, the medium was replaced with DMEM (Hyclone) containing 10 % fetal bovine serum (Gibco, Invitrogen). After transfection, the cells were harvested at 48 h for RNA or protein extraction.

Statistical analysis

DPSCs were seeded at 0.7×10^4 cells/well into 6-well plates in triplicate for each experimental condition. OSM (10 ng/ml, Sigma, human, recombinant) was added to the cultures or not. DPSCs were collected at 12, 24, 48, 72 h after plating and dissociated. The total cell numbers were counted and the results were expressed as mean $\pm$ SEM. Data are expressed as mean $\pm$ SEM of results of three or more independent experiments. The significance of differences between the experimental groups and controls was analyzed using ANOVA. Statistical significance was evaluated by the independent samples t-test using SPSS v17.0 software. Statistical significance was defined as $P < 0.05$.

Results

Effect of OSM in osteogenic differentiation of DPSCs

To explore if OSM affects osteogenic differentiation of DPSCs, we cultured DPSCs in osteogenic

differentiation medium in the presence of different concentrations of OSM, and ALP expression was increased. As shown in Fig. 1, exposure of DPSCs to osteogenic differentiation medium for 7 days, but not to normal medium, we found that ALP expression was increased and OSM treatment increased the ALP activity of DPSCs with the increased concentration of OSM (Fig. 1a, b). Moreover, when DPSCs were cultured under the same conditions and stained with Alizarin red, similar staining was observed (Fig. 1c, d). In addition, with the differentiation of DPSCs, we found that their ALP activity was increased when DPSCs were treated osteogenic medium and OSM together (Fig. 1e). These results indicated that OSM has the potential to contribute to osteogenic differentiation of DPSCs and this phenomenon is time and concentration dependent.

Oncostatin M (OSM) promotes osteogenesis marker genes expression of DPSCs

In order to investigate the influence of OSM on osteogenesis marker genes expression of DPSCs, DPSCs were treated with OSM during differentiation. Western blotting analysed showed that OSM significantly increased osteogenesis marker genes expression, including alkaline phosphatase (ALP), osteocalcin (OCN), bone morphogenetic protein 2 (BMP2) and Runt-related transcription factor 2 (Runx2) (Fig. 2a). And these markers' mRNA expression of these markers was significantly increased when cultured in osteogenic medium in the presence of OSM (Fig. 2b–e) ($*P < 0.05$).

OSM does not interfere with the proliferation of DPSCs

Furthermore, we evaluate whether the changes of osteoblast markers' expression was due to OSM induced proliferation of DPSCs. The proliferation ability of DPSCs was assessed by cell counting assays and MTT analysis during early time-points of differentiation was investigated. The cell number was determined between DPSCs and OSM-treated DPSCs, which showed no significant difference. The number of DPSCs treated with OSM at the end of 72 h was similar to that of untreated DPSCs (Fig. 3a). MTT analysis showed no statistically significant changes in

Fig. 1 Effect of OSM in osteogenic differentiation of DPSCs. **a** DPSCs were cultured for 7 days in the presence or absence of osteogenic differentiation medium containing different concentration of OSM, and stained with ALP. **OC** osteogenic condition. **b** Quantification of ALP activity by staining ($n = 3$, $P < 0.05$, the *asterisk* indicates significant differences from the control groups). **c** DPSCs were cultured for 14 days in the presence or

absence of osteogenic differentiation medium (OC) containing different concentration of OSM, and stained with Alizarin red S. **d** Quantification of Alizarin red staining. ($n = 3$, $P < 0.05$, the *asterisk* indicates significant differences from the control groups). **e** Relative ALP staining (relative to GAPDH expression). ($n = 3$, $P < 0.05$, *asterisk* significantly different from the control groups)

the early time of differentiation (Fig. 3b). Our results can be concluded as follows: After OSM stimulation, Alizarin red staining and elevated expression of ALP did not correlate with the cell number any more.

Effect of JAK3 inhibitor on osteogenic differentiation of DPSCs induced by OSM

Previous studies have shown that the effects of OSM on osteogenic differentiation of bone marrow mesenchymal stem cells are mediated by activation of JAK3/STAT3 signaling pathway (Song et al. 2007).

Therefore, we further studied the roles of JAK3/STAT3 signaling pathway in OSM promotes the differentiation of DPSCs into osteoblasts. DPSCs were differentiation for 7 days, and treated with a specific inhibitor of JAK3, WHI-P154, we found that 50 μ M WHI-P154 could decrease ALP activity even when treated with OSM (Fig. 4a, b) ($*P < 0.05$). OSM increased the activation of STAT3 in DPSCs and this activation was dose-dependent inhibited by treatment with WHI-P154 (Fig. 4c). WHI-P154 also inhibited osteoblast markers' mRNA expressions (Fig. 4d–g) ($*P < 0.05$).

Fig. 2 Oncostatin M (OSM) promotes osteogenesis marker genes expression of DPSCs. DPSCs were cultured for 7 days in the presence or absence of osteogenic differentiation medium (OC) containing OSM (10 ng/ml) or not. **a** ALP, OCN, RUNX2 and BMP2 expression was detected by Western blot. **B–E** Quantitation of PCR products. The quantity of the amplified products was analyzed by an image analyzer. ($n = 3$, $P < 0.05$, the asterisk indicate significant differences from the control groups)

JAK3/STAT3 knockdown inhibits OSM-induced osteoblastic differentiation of DPSCs

To determine the molecular mechanism of JAK3/STAT3 signaling pathway, we knockdown JAK3 by using siJAK3. The expression of JAK3 was significantly inhibited (Fig. 5a) ($*P < 0.05$). On the other hand, knockdown of STAT3 using siRNA almost completely inhibited STAT3 activities (Fig. 5b) ($*P < 0.05$). Seven days after differentiation of the ALP activity was decreased when treated with siJAK3 or siSTAT3, whereby the ALP activity decreased

significantly for the siSTAT3 treated group (Fig. 5c, d) ($*P < 0.05$). The mRNA expression of osteoblast markers, also decreased (Fig. 5e–h) ($*P < 0.05$). The above results showed that JAK3/STAT3 signaling pathway plays an important role in OSM induced osteoblastic differentiation of DPSCs.

Discussion

In this study, we first demonstrated that OSM regulates lineage-specific differentiation of DPSCs for

Fig. 3 OSM does not interfere with the proliferation of DPSCs. **a** DPSCs were treated with OSM (10 ng/ml) for 12, 24, 48 and 72 h, and taken count of the number of cells. No significant differences in cell proliferation after OSM treatment were

observed. **b** Cell proliferation of DPSCs was analysed by the MTT assay 12, 24, 48 and 72 h after stimulation with 10 ng/ml OSM stimulated by the MTT assay

osteoblast. More and more evidences indicate that IL-6 and LIF are involved in the regulation of bone formation and bone remodeling (Taguchi et al. 1998; Heymann and Rousselle 2000). OSM is a member of the interleukin family, and in terms of osteogenic differentiation of DPSCs, OSM should have similar effects. Also, OSM may be a protective factor in human articular cartilage injury. OSM can activate osteoblasts and inhibit the absorption of bone, so there is a strong osteogenic regulatory effect. (Bellido et al. 1998; Chipoy et al. 2004; Malaval et al. 2005). Recent studies have indicated that OSM can maintain bone formation by means of OSM Receptor (OSMR) and STAT3, which acts on the recruitment, proliferation and osteogenic differentiation of mesenchymal progenitor cells (Guihard et al. 2015). OSM can stimulate the growth of endothelial cells, fibroblasts and osteoblasts, which indicates that OSM may be a key regulator of the marrow microenvironment, which is helpful to the formation of bone (Hoermann et al. 2011). These studies support our present finding that OSM has a potent stimulatory effect on the osteogenic differentiation of DPSCs.

OSM has been shown to have a major role in bone metabolism (Malik et al. 1995; Jay et al. 1996; Walker et al. 2010). While hBMSCs were not sensitive to OSM in the resting state without stimulation, OSM induced ALP activity and ALP mRNA after the OSM treatment. This might be due to the expression levels of OSMR and gp130, since they are rapidly induced after OSM stimulation in mouse pre-adipocyte 3T3-L1 cells (Miyaoaka et al. 2006). Furthermore, the current study showed that OSM promotes osteoblastogenesis and suppresses adipogenesis, not only in hBMSCs but

also in mouse C3H10T1/2 cells. OSM appears to be a potential master regulator of MSC lineage allocation, since it was able to shift MSCs from the adipogenic to the osteoblastic lineage, regardless of cell specificity or adipogenic stimulant.

On the one hand, OSM is involved in embryonic development, differentiation, inflammation and regeneration of various tissues, mainly in the liver, bone, central nervous system and hematopoietic system expression; on the other hand, it inhibits the growth of tumor cells, such as inhibition of colon cancer, ovarian cancer, breast cancer, neurological glial tumor and lung cancer cell proliferation. Onco-statin binds to its receptor and activates Jak1, Jak2 and Tyk2, leading to phosphorylation of the STAT family, in particular of STAT3. Changes in cell morphology and cell motility induced by oncostatin also depend on the state of STAT3. OSM has been known to activate the JAK/STAT in many kinds of cells (Gomez-Lechon 1999). According to several records, OSM induces JAK1 and JAK2 phosphorylation and activation of STAT2 and STAT3 in vascular smooth muscle cells, and in vascular smooth muscle JAK3 was activated by OSM signaling pathway (Bernard et al. 1999; Nagata et al. 2003; Demyanets et al. 2011). Furthermore, OSM can activate JAK2, JAK3, and stimulate the osteogenic differentiation of human adipose mesenchymal stem cells (hADSCs), increase the expression of osteogenic differentiation markers, such as ALP, RUNX2, osteocalcin and so on (Song et al. 2007). In addition, OSM is secreted by activated peripheral blood mononuclear cells and induces bone marrow mesenchymal stem cells into osteoblasts through a mechanism of the STAT3 (Guihard et al.

Fig. 4 Effect of JAK3 inhibitor on osteogenic differentiation of DPSCs induced by OSM. DPSCs were cultured with or without WHI-P154 at the indicated concentrations for 5 h and then treated for 15 min with OSM (10 ng/ml). **a** DPSCs were cultured in osteogenic differentiation medium containing OSM (10 ng/mL) for 7 days and treated with or without WHI-P154 and were then stained with ALP. **b** Quantification of ALP staining in cultures detailed in **a** ($n = 3$, $P < 0.05$, asterisks indicate significant differences from the control group). **c** Cell lysates were analyzed with Western blot using antibodies recognizing phospho-STAT3 (p-STAT3) and total STAT3. **d–g** Quantitation of PCR products of ALP, OCN, Runx2 and BMP2. The quantity of amplified product was analyzed by an image analyzer. ($n = 3$, $P < 0.05$, asterisks indicate significant differences from the control group)

2012). Based on these results we can deduce that in the osteogenic differentiation induced by OSM, JAK and STAT3 signals play an important role.

In present study, OSM and JAK/STAT3-induction have positive effects on the mineralization of DPSCs, increase expression of the osteoinductive growth factor BMP2, and the osteogenic marker ALP, OCN and Runx2, which can be blocked with WHI-P154. OSM did not affect cell number. We conclude from

these findings that OSM promotes the differentiation of DPSCs into osteoblastic lineages and increases mineralization. On the other hand, IL-6-type cytokines can activate the JAK/STAT pathway to promote osteoblast differentiation (Bellido et al. 1998). Activation of JAK/STAT3 by IL-6, including OSM, contributed to the activation of signal transduction pathways in bone cell differentiation by increased ALP activity, and osteocalcin expression was

Fig. 5 JAK3/STAT3 knockdown inhibits OSM-induced osteoblastic differentiation of DPSCs. **a–b** 24 h after siRNA introduction, expression of JAK3, STAT3 was analyzed by Western blot. **c–d** Effects of knockdown of JAK3 or STAT3 on OSM induced ALP activities in DPSCs. 24 h after siRNA introduction, DPSCs were cultured for additional 7 days under the indicated conditions. OSM was utilized at 10 ng/mL. ALP activities were measured, normalized to cellular protein contents, and are presented as mean $\pm$ SEM (n = 3). * P < 0.05 versus the control siRNA-transfected cultures with OC (Osteogenic condition) and OSM. **e–h** Effects of knockdown of JAK3 or STAT3 on DPSCs. 24 h after siRNA introduction, effects of knockdown of JAK3 or STAT3 on mRNA expression of ALP, OCN, BMP2 and Runx2 in DPSCs (RT-PCR analysis). (n = 3, P < 0.05, asterisks indicate significant differences from the control groups)

demonstrated in vitro (Li 2013). These results suggest that STAT3 plays a crucial role in the differentiation of DPSCs.

In summary, there are a variety of biological activities of OSM in vivo, the present research still continues to reveal these features. The interaction between OSM and other cellular factors in the process of network regulation is yet to be further studied. Here we presented a treatment mechanism of OSM-mediated differentiation of DPSCs through the JAK3/STAT3 pathway. In the future, OSM can be a possible

therapy based on the osteogenic differentiation of DPSCs, and in view of clinical applications it is necessary to verify whether OSM is involved in other biological functions of DPSCs.

Conclusion

With the increasing demand for a high quality of life, the high development of tooth tissue engineering is concerned. DPSCs play an important role in tissue

regeneration. Using DPSCs to realize the regeneration of alveolar bone is of practical significance. Studies on the differentiation of DPSC are still in the primary stage and its regulation mechanism is still not clear, there are many open questions. The biological activities of OSM in the body is multifarious. In stem cells, OSM has a strong capacity to regulate bone formation. Dental pulp stem cells provide a new direction for future research in tissue engineering. OSM and its antagonists in the regeneration of alveolar bone have a promising future.

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